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Neuronal Communication: Firing Spikes with Spikes

Spikes of single cortical neurons can exert powerful effects even though most cortical synapses are too weak to fire postsynaptic neurons. A recent study combining single-cell stimulation with population imaging has visualized *in vivo* postsynaptic firing in genetically identified target cells. The results confirm predictions from *in vitro* work and might help to understand how the brain reads single-neuron activity.

Michael Brecht

One summer holiday in the early seventies, I lost my mind to a slot machine. Like a little robot I dropped penny by penny into a so-called penny fall (Figure 1A), being absolutely certain with the next penny a huge avalanche of copper coins would fill my pockets and finance our family holiday. It never happened. My parents cut the penny supply line as I failed to grasp that the machine was adjusted such that it would spit out fewer pennies than I inserted and that the probability to fall for any one penny in the machine was very low. In this issue of *Current Biology*, Kwan and Dan [1] report how they played a similar low-return game in mouse visual cortex [1]. They dropped spikes into single neurons and looked out for spike firing returned by the cortical network. It happened (Figure 1B). The spike return in response to spike insertion is of considerable interest, because of its implications for cortical processing [2].

Critical to Kwan and Dan's [1] experimental success was their ability to sample large numbers of cells by imaging and the fact that they imaged mouse lines in which specific cell populations express fluorescent proteins. In this dispatch I shall briefly consider: firstly, why the prior of observing postsynaptic spiking in response to firing a single cortical neuron is low; secondly, exceptions to this rule; thirdly, the evidence that single neurons can powerfully impact on brain activity anyway; and lastly, what such results may tell us about cortical network organization.

As a Rule Cortical Synapses Are Weak

The study of synaptic transmission in vertebrates was pioneered in the muscle nerve preparation, where a single motor neuron action potential might evoke a 70 mV depolarization and will result in an action potential in the postsynaptic muscle [3]. Synaptic connections between cortical neurons turned out to be quite different, however. Even though they often involve multiple synaptic terminals, unitary connections are on average much weaker, with an average postsynaptic depolarization of around 1 mV [4]. This is much less than the 10–40 mV required for bringing the postsynaptic neuron to firing threshold. Indeed, in dual intracellular recordings from synaptically connected pyramidal cells in brain slices — the classic preparation for studying cortical synaptic transmission — monosynaptically evoked postsynaptic firing is exceedingly rare and signs of polysynaptic activation are typically absent.

Exceptions to the Rule

The weak average strength of cortical synapses makes perfect sense in light of the thousands of synapses made by cortical neurons [5]. Given this large number of postsynaptic targets, it is critical to consider not only the (weak) average strength of cortical synapses but also the distribution of synaptic strength. It turns out that

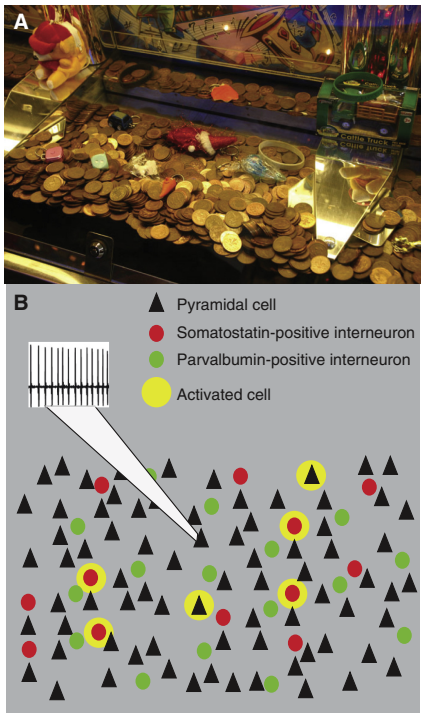

Figure 1. Dropping pennies into slot machines and spikes into cortex: what's the return?

(A) A penny fall is a slot machine, in which one inserts pennies in order to make other pennies drop and return to the gambler. Even though it looks like many pennies are about to fall, the probability to drop is very low for any one penny. Overall the machine returns fewer pennies than inserted. (Credit: StockphotoPro). (B) The spike insertion experiment of Kwan and Dan [1]. Spikes (top left) were inserted via patch pipettes (white) into single neurons of mouse visual cortex. The authors assessed spiking across many cells by a fluorescence increase of a calcium-dye (yellow circle). While the probability of spiking in response to single cell stimulation is very low for pyramidal cells (black) and close to zero for parvalbumin-positive interneurons (green), a sizeable fraction of somatostatin-positive interneurons (red) is activated. The overall spike return of cortex in response to single cell stimulation remains to be determined.

synaptic strength between neurons is fairly heterogeneous and follows a log-normal distribution, with many weak synapses but also a few strong ones [6]. The impact of these few strong synapses on network activity is poorly understood, but they obviously are one mechanism by which single cell stimulation can activate postsynaptic cells [7].

Another way by which single neurons could activate postsynaptic cells is through a burst of presynaptic spikes combined with postsynaptic summation and facilitation. In the typical pyramidal-to-pyramidal connection this trick will not work, however, because these synapses depress upon repeated activation. Pyramidal cells also connect to specific cortical interneurons and some of these connections have the summation and facilitation properties required for postsynaptic activation. Somatostatin-positive interneurons known as Martinotti-cells show tremendous facilitation, and *in vitro* work demonstrated that these cells can be robustly activated by repeated activation of single cortical inputs [8–10]. Not surprisingly then, somatostatin-positive neurons showed prominent postsynaptic activation in the imaging experiment of Kwan and Dan [1].

When I said earlier that signs of polysynaptic activation are typically absent in dual recordings from cortical neurons, this was not entirely true. This result is robust in brain slices from rodents *in vitro.* It was also shown that firing single spikes in cortical neurons in rodents *in vivo* leads to measurable but also comparatively weak network effects [2]. Oddly enough, however, human cortical neurons seem to sing a different tune. In brain slices from human cortical tissue that was extracted during cancer surgery, Tamas and colleagues [11] observed in a large fraction of cases polysynaptic activation patterns following single spike activation. How the human brain can properly function when single spikes trigger a barrage of postsynaptic spiking events remains a mystery.

Single Neuron Impact on Brain and Behavior

If cortical synapses are typically weak, why bother about the impact of single neuron stimulation in the first place? A variety of experiments have shown that activity of single cortical cells can have more impact on brain and behavior than one might initially be inclined to believe. Cortical activity is fairly sparse and spikes stand out against a background of relatively silent neurons [12]. Thus, it was shown that activation of single cortical neurons in the rat vibrissa motor cortex could evoke long sequences of small whisker movements [13] and that stimulation of single cells in the rat's somatosensory cortex affected behavioral report in a detection task [14]. In slices it was shown that certain types of interneurons could kick off huge population events [15]. Finally, Dan's group showed in an earlier study [16] that intense activation of single cortical neurons could lead to global changes of network activity and brain state.

What Do the Current Results Tell Us about How the Cortex Works?

The capacity of the cortical network to translate spikes in single neurons into changes in behavior is astonishing given the vast number of cortical neurons. To date we have had only very little information on the downstream synaptic events that mediate this capacity. In the data of Kwan and Dan [1], the activation of parvalbumin-positive interneurons, which target somata and axon hillocks, is conspicuously absent. The parvalbumin cells are in a position to prevent excessive network activation, but this microcircuit does not seem to be recruited by single cell stimulation.

Another finding, mentioned above, is the activation of the somatostatin-positive interneurons: the authors [1] estimate that burst firing of one pyramidal cell may activate 30% of these cells (~3–9 cells) locally. These putative Martinotti-cells target dendrites and dendritic inhibition can dramatically reduce dendritic calcium-spikes and cellular bursting behavior [17]. If I had to make a guess, I would say that we see here a burst-sparsifier in action, where the cortex detects intense activity in single cells and then curbs down dendritic excitability and bursting in widespread networks. Because dendritic excitability might be critical for synaptic plasticity, this mechanism may restrict learning to a few intensely activated cells.

Finally, Kwan and Dan [1] see a small fraction of pyramidal cells (~15) return spikes to single cell burst. Most of these cells are only weakly activated, but they are nonetheless strong candidates for long-range signaling following single cell bursts. It would be most interesting to characterize their synaptic inputs and see if they indeed receive detonator synapses from the activated neuron. The question how the brain reads its own activity is a profound one. Data such as those provided by Kwan and Dan [1] are an important step towards confronting this issue.

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Cell Polarity: Stretching Prevents Developmental Cramps

Initiation and successive development of organs induce mechanical stresses at the cellular level. Using the tomato shoot apex, a new study now proposes that mechanical strain regulates the plasma membrane abundance of the PIN1 auxin transporter, thereby reinforcing a positive feed-back loop between growth and auxin accumulation.

Hongjiang Li, Jiří Friml, and Wim Grunewald

Coordinated cell and tissue polarization is crucial during both plant and animal development and requires an elaborate control system with multiple feed-back mechanisms. In plants, the polar localization of the PIN auxin transporters is pivotal for the directional transport of the signaling molecule auxin [1]. This transport is responsible for the generation of auxin gradients, which then trigger specific molecular programs to regulate organogenesis in response to developmental and environmental cues [2]. Auxin itself feeds back on tissue and organ polarity, through transcriptional and post-translational mechanisms regulating PIN localization [3,4]. Besides this physiological control, plant morphogenesis is also regulated by the mechanical properties of individual cells. Microtubules, dynamic components of the cytoskeleton, form an ordered cortical array within the cell. In growing plant cells, this array is typically oriented transverse to the growth direction, allowing growth in one direction while restricting it in other directions [5]. An extra level of mechanical constraint is exerted by the cell wall. Unlike animals, plant cells are encaged by cell walls, which they share with their neighbors. Interestingly, the presence of these neighboring cells has been shown to affect microtubule organization [6]. Growth and division of cells that are glued to each other thus induce considerable mechanical stresses on both the cellular and tissue level.

Recently, it was shown in the *Arabidopsis* shoot apical meristem that microtubules reorient upon mechanical stress [7]. This provided a paradigm in which mechanical signals, triggered by the growth of an organ, feedback on microtubule orientation and thus morphogenesis. Moreover, it was found that the orientation of subcortical microtubule arrays is highly correlated with PIN1 polarity [8], and computer models predicted that PIN1 proteins would preferentially localize to plasma membrane regions with the highest mechanical strain [8]. However, experimental evidence for the impact of mechanical stress on PIN-mediated auxin transport was so far missing.

Using osmotic treatments, external force applications, membrane modulations and growth induction, Nakayama and colleagues [9] report in this issue of *Current Biology* that growth-induced mechanical strain upregulates PIN1 function and auxin accumulation in the tomato shoot apex. These findings thus add another layer of feedback on coordinated plant growth and development, i.e. growth-induced mechanical stresses that promote auxin-mediated growth.

At the plasma membrane region with the highest mechanical tension, Nakayama *et al.* [9] observed an increased PIN1–GFP signal. However, since the established PIN1 polarity was not altered, it seems that mechanical strain affects PIN1 abundance at the predefined polar domains rather than PIN polarity *in se*. The authors hypothesize that their findings are most probably achieved by a general increase of exocytosis and reduced endocytosis. This would imply that the cellular response to mechanical stress is a universal phenomenon for all recycling plant plasma membrane proteins. Although the putative involvement of intracellular trafficking integrates the role of mechanical stress into the current understanding